Abstract A5.8 Table 1

	Mean DAS28 at baseline	Mean DAS28 at 3 months	Mean DAS28 at 6 months	Goodeular response at 3 months	Goodeular response at 6 months	DAS28 < 2.6 at 3 monts	DAS28 < 2.6 at 6 months	HAQ at baseline	HAQ at 3 months	HAQ at 6 months
RF +	5.73 ± 1.24	3.38 ± 1.30	4.13 ± 1.83	90.5%	65.2%	18.2%	17.4%	1.81 ± 0.77	1.00 ± 0.57	1.2 ± 0.74
RF –	5.52 ± 1.61	3.72 ± 0.93	4.22 ± 1.23	83.3%	57.1%	14.3%	14.3%	1.57 ± 0.78	0.83 ± 0.56	0.95 ± 0.54

Objective To evaluate the efficacy of RTX in our series of refractory seronegative and seropositive RA.

Materials and Methods Baseline characteristics and disease activity markers at baseline, and after 3 and 6 months of treatment with RTX (1g × 2 weeks), were collected in 33 patients. A descriptive study was made; and the relations between variables were analysed statistically.

Results The mean age was 52.06 ± 12.01 years, 75.8% female, 78.8% RF positive. The mean duration of illness was 7.70 ± 4.47 years. Thirty two patients (97%) had failed at least to one TNF antagonist. Most of the patients (84.8% 9) received RTX with methotrexate.

The mean DAS28 at baseline was 5.7 ± 1.30; at 3 months decreased to 3.4 ± 1.22, and at 6 months to 4.15 ± 1.69 ($p < 0.0005$).

At 3 months, 88.9% reached good eular response, and 63.3% at 6 months. Remission was obtained in 17.2% at 3 months and in 16.7% at 6 months.

It was also noted improvement in baseline HAQ, after 3 and 6 months (from 1.75 ± 0.767 to 0.96 ± 0.56 and 1.24 ± 0.70 respectively).

No significant differences were found between decreases in DAS 28 at 3 and 6 months compared to baseline between RF seronegative and seropositive patients, neither in good eular response, remission percentages or HAQ improvement. The data are shown in the table.

Discussion The efficacy and safety of RTX has been proved in several clinical trials.

The presence of RF, low baseline functional disability and no more than one previous anti-TNF are predictors of good response to RTX, as has been recently published.

Response rates in seronegative RA are slightly lower, although higher than placebo, as described in other publications.

In conclussion, the experience of RTX treatment in our patients with seronegative RA is positive, in terms of efficacy, due to the action on B cells and their different roles, with no significant differences comparing to seropositive RA.

A5.9 VALUES OF B LYMPHOCYTE SUBPOPULATIONS (HEALTHY POPULATION) USING FLOW CYTOMETRY A STARTING POINT FOR ANY STUDY

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Background Flow cytometry is a widely used technique nowadays for the determination of cell subpopulations in the study of autoimmune, infectious and tumoral diseases.

Our goal is to study the means and medians of B lymphocyte subpopulations in healthy population, to thereby have reference limits with which being able to compare when carrying out studies in population with autoimmune diseases.

Methods We studied 50 healthy patients. Male/female: 25/25. Age: 18–65 years. Previously, it was checked that none of these patients had any autoimmune diseases nor any other disease or condition causing lymphopenia or lymphocytosis. The fluorochromes used were: CD3, CD19, CD38, CD27 and IgD.

Results

Abstract A5.9 Table 1

Subpopulations	Mean	Typical deviation	Median
Naive	70.46	8.06	73.70
DN	4.05	2.08	4.66
Unswitched Memory	9.65	5.33	10.05
switched Memory	11.38	4.62	11.00
Bm1	7.82	9.29	5.10
Bm2	73.14	10.71	73.05
PreGC	2.90	1.61	2.89
Bm3 + 4	0.56	0.53	0.44
Late Bm5	3.17	2.74	2.50
Early Bm5	9.12	4.93	9.21

Conclusions Knowing the mean, medians and standard deviations of the B lymphocyte subpopulations subsets is important in helping to compare these results with those obtained in studies of patients with autoimmune diseases.

In most cases, conclusions drawn after the study with flow cytometry are based on knowing how these subpopulations vary with respect to healthy people in order to draw conclusions about what subpopulations are involved the most in the pathogenesis of the disease.

We believe, therefore, important to deepen in studies of this kind in order to clarify more situations of normality in the world of flow cytometry: a technique that is increasingly taking more importance in the understanding of autoimmune diseases.

A5.10 Δ4BAFF ALTERNATIVE SPLICING IS REGULATED BY IFN-γ AND SC35 PROTEIN

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Background The B-cell activating factor (BAFF) is a potent survival factor involved in the pathogenesis of autoimmune diseases. Recently, we reported the discovery of a new transcript for BAFF, Δ4BAFF – lacking exon 4 –, which is mainly detected in autoimmune diseases and acts as a transcription factor for its own gene. However, the mechanisms implicated in Δ4BAFF induction and upregulation are unknown. In this study we analysed the induction and regulation of Δ4BAFF.

Materials and Methods First, to study the alternative splicing of *BAFF* exon 4, we transfected a minigene construct, centred on exon 4, into RAMOS B cells. To determine the proteins implicated in exon 4 inclusion/exclusion, we co-transfected the minigene together with each of the plasmids coding for the main splicing proteins

(SC35, SRp40, SRp55, SRp20 and hnRNPA1), and the ratios between exon4 inclusion/exclusion were evaluated by RT-PCR. Second, we examined the effects of different cytokines on Δ4BAFF induction.

Results RAMOS cells presented exon 4 skipping (ratio inclusion/exclusion: 6.8) after minigene transfection. Following co-transfection of the minigene with coding plasmids for splicing proteins, only the overexpression of SC35 showed effect in the splicing of exon 4, promoting exon 4 inclusion (ratio: >30). Incubation of different cell lines with several cytokines showed that IFN-γ was able to induce Δ4BAFF-transcript. Thus, after IFN-γ stimulation in the minigene model, the ratio inclusion/exclusion markedly decreased (1.5). IFN-γ modifies the balance between SC35 and another member of hnRNPs family (hnRNP C1/C2) favouring the alternative splicing of exon 4.

Conclusions These results demonstrated that IFN-γ induces Δ4BAFF, modifying the function of SC35 protein and increasing the hnRNPC1/C2. Our study provides an expanded conceptual view of BAFF gene regulation, and contributes to a better understanding of the mechanisms involved in BAFF up-regulation in autoimmunity.

A5.11 DETECTION OF ACPA PRODUCING B-CELLS BY A CITRULLINE PEPTIDE PANEL

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Background and Objectives Anti-citrullinated protein/peptide antibodies (ACPAs) are the most sensitive and specific serological markers of RA. To identify the optimal epitopes that detect different subgroups of RA patients with high sensitivity and specificity, we have investigated citrulline and arginine containing peptides derived from filaggrin, collagen or vimentin. We have identified a citrulline-containing peptide panel that was recognised by RA sera with high specificity. Our aim was to compare this peptide panel with the conventionally used serological assays and to detect peptide-specific ACPA producing B-cells in in vitro cultures.

Materials and Methods Previously selected citrulline- and arginine-containing filaggrin, vimentin and collagen peptide epitopes were investigated. We compared the recognition of these peptides by RA and control sera using indirect ELISA. B-cells were purified from peripheral blood by negative selection, IgG production was stimulated by B-cell activators (R848 and recombinant human IL-2) provided with the human ELISPOT kit. Antibody producing cells were enumerated after 4 days culture by using peptide-specific ELISPOT assay.

Results Sera samples from 247 RA and 148 age-matched (57 ± 14 years) healthy controls were collected. The citrulline peptide panel detected approximately 80% of RA patients, including 20% of seronegative/CCP negative patients as well. Individual peptides detected different subgroups of RA patients. The more peptides recognised by a particular RA serum sample, the more severe the disease of the patient was. In vitro cultured B-cells from selected RA patients synthesised multiple citrulline-containing peptide-specific antibodies after polyclonal stimulation, while B-cells from healthy blood donors did not.

Conclusions The citrulline peptide panel can detect 20% of ACPA negative RA patients thus may have a prognostic value. Furthermore, the panel is suitable to detect citrulline peptide-specific antibody producing cells, thus enables us to study ACPA producing B-cells of RA patients.

A5.12 DISAPPEARANCE AND REAPPEARANCE OF IGG, IGA AND IGM AUTOANTIBODY ISOTYPES AND IMMUNE COMPLEXES IN RITUXIMAB-TREATED SLE PATIENTS

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Background and Objectives We have earlier investigated the content of specific IgG autoantibodies in SLE immune complexes (IC; Åhlin *et al*, Lupus 2012; 21:586). For that purpose we have developed a line blot technique for the quantification also of non-classical (IgA and IgM) SLE autoantibody isotypes. In SLE patients, IgG autoantibody levels drop after institution of Rituximab therapy. The objective was to investigate parallel changes IgG, IgA and IgM autoantibody isotypes in parallel to IC levels.

Materials and Methods Nine SLE patients initially treated with two infusions of Rituximab were followed with repeated samplings at baseline and after 1, 3, 6 and 12 months. Thawed samples were investigated simultaneously concerning rheumatoid factor (RF) isotypes and C1q-binding IC with enzyme immunoassays. All samples from patients with ANA-associated autoantibodies (6/9) were investigated concerning IgG/A/M autoantibodies with line blot quantitated with densitometry and concerning IgG autoantibodies with ALBIA/Luminex technique. Significant changes were defined either as ≥33% drop or as ≥50% increase, compared to the lowest levels experienced during the follow-up period.

Results ALBIA measurements showed significant initial drop in anti-dsDNA in 4/6 patients but also significant drop in levels of anti-histone, anti-SSA/Ro60, anti-Sm and anti-Sm/RNP in individual patients. Late increases in IC and antibodies against dsDNA, SSA/Ro52, SSA/Ro60, SSB, Sm, Sm/RNP ribosomal P protein and histones were associated with clinical relapse. Late increase in IgA/IgM anti-DNA, anti-histones and anti-nucleosomes was also found in one patient with persistent kidney disease treated with mycophenolate mofetil at 10 months. Non-classical autoantibody isotypes showed late increases that often were not paralleled by the corresponding IgG autoantibodies. Two patients showed late increase in RF isotypes in parallel to clinical relapse. Different autoantibodies/isotypes showed different kinetics of appearance/disappearance. All ANA autoantibody positive patients initially had increased IC levels, which dropped significantly after therapy in 4/6 patients. The autoantibody negative patients never had increased IC levels and showed no significant changes in RF.

Conclusions Measurement of non-classical isotypes of RF and ANA-associated autoantibodies might yield clinically useful information when monitoring SLE patients treated with B cell depleting therapy.

A5.13 EFFECT OF RITUXIMAB ON B CELL SUBPOPULATIONS EXPRESSING THE 9G4 IDIOTYPE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background and Objectives Antibodies encoded by the V_H4–34 gene are inherently autoreactive, binding to red blood cell